β -ENOLASE, CREATINE KINASE M AND B, AND S-100 α_0 CONCENTRATIONS IN THE MASTICATORY AND LINGUAL MUSCLES OF RATS

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ABSTRACT

The concentration of proteins per mg total soluble protein in the lingual muscle of rats was $20.0 \pm 2.4 \mu\text{g}$ (mean $\pm$ SD) for β -subunit of enolase (β -enolase), $33.2 \pm 2.7 \text{ ng}$ for $\alpha\alpha$ -form of S-100 protein (S-100 α_0), $54.3 \pm 4.0 \mu\text{g}$ for M-subunit of creatine kinase (CKM), and $900.5 \pm 13.8 \text{ ng}$ for B-subunit of creatine kinase (CKB). The concentrations in the masseter, temporalis and digastric muscles were widely different from those in the lingual muscle, but were relatively similar to each other; $31.1\text{--}45.2 \mu\text{g}$ for β -enolase, $12.4\text{--}17.1 \text{ ng}$ for S-100 α_0 , $147.1\text{--}181.3 \mu\text{g}$ for CKM, and $187.5\text{--}282.5 \text{ ng}$ for CKB. In the diaphragm, which is intermediate, in terms of fiber type composition and contractile property, between the fast (EDL) and slow (SOL) muscles in the hindlimb, the concentrations of β -enolase ($34.3 \pm 1.6 \mu\text{g}$), CKM ($98.8 \pm 3.8 \mu\text{g}$), and CKB ($255.8 \pm 8.3 \text{ ng}$) were intermediate between the EDL and SOL muscles. However, the concentration of S-100 α_0 ($16.4 \pm 1.9 \text{ ng}$) were extremely low. The results indicate that expression of different muscle proteins is controlled differentially in individual muscles and is not necessarily correlated with the conventional classification of fiber types.

Recently we have developed immunobiochemical systems in rats to determine the concentration of several muscle proteins; β -subunit of enolase (β -enolase) (15), S-100 protein composed of two α -subunits (S-100 α_0) (11), M-subunit of creatine kinase (CKM) (14), and B-subunit of creatine kinase (CKB) (16). They are distributed predominantly in the cytoplasm, but differentially over fast (extensor digitorum longus; EDL) and slow (soleus; SOL) muscles in the hindlimb (19, 20). The concentration of these proteins in these muscles changes in a characteristic manner following sciatic nerve transection, following sciatic nerve reunion performed immediately after transection, and also following cross-reunion of the nerves innervating the SOL and EDL muscles (19, 20). In some pathological processes, those proteins appear in the blood (22, 23, 27, 31).

In the present investigation, we determined the

normal concentration in the lingual and masticatory (masseter, temporal and digastric) muscles of four muscle proteins; β -enolase, S-100 α_0 , CKM, and CKB. Measurement was also made for the EDL and SOL muscles, and at the same time for the diaphragm which is, in terms of fiber type composition and contractile property, intermediate between the EDL and SOL muscles (21).

MATERIALS AND METHODS

Eight male Wistar rats of about 350 g body weight were deeply anesthetized with chloroform. After decapitation the bilateral masticatory muscles (masseter, temporalis and digastric), the lingual muscles, the bilateral EDL and SOL muscles, and the diaphragm were sampled on ice and stored at -80°C for a few days before measurement. The muscle tissues were homogenized with a glass

Fig. 1 Concentrations per mg soluble protein (mean and standard deviation) of β -enolase, S-100a₀, CKM and CKB in the muscles in the oral region (white columns) as compared to those in the hindlimb muscles (hatched columns) and diaphragm (dotted columns). N=8, for the lingual muscle and the diaphragm; and N=16, for other muscles

homogenizer in 10 vol of 50 mM Tris-HCl buffer, pH 7.5 containing 5 mM MgCl₂. The homogenates were centrifuged at 20,000 *g* at 4°C for 20 min. Enolase and creatine kinase are the cytoplasmic enzymes, and they are extractable under our conditions (14, 15). A portion (<10%) of S-100 protein is reported to be present in a membrane-bound form (26). However, most of S-100 protein in muscle tissues is extracted under our conditions (13). The soluble fraction was used for the assay of muscle proteins. Protein concentration in the extract was determined by the dye-binding method of Bradford (1). The concentrations of β -enolase, S-100a₀, CKM and CKB were determined with the sandwich-type enzyme immunoassay systems described previously (11, 14–16). In brief, the immunoassay system consisted of polystyrene balls with immobilized rabbit antibodies monospecific to the respective subunits of rat CK, enolase, or human S-100 protein, and the same antibodies labeled with β -D-galactosidase from *Escherichia coli*. Each assay was specific to the respective subunits of isoproteins, and the minimum detection limit of the assay was 1 to 3 pg per assay tube. The assay for S-100a₀, CKM, CKB, and β -enolase was performed with purified human S-100a₀, rat CK-MM, rat CK-BB and rat β -enolase, respectively, as standards, and the results

were expressed as ng or μ g of the respective homodimeric isoproteins equivalent per mg soluble protein.

RESULTS

The concentration of β -enolase (μ g/mg protein) was high in the EDL muscle (66.0 ± 2.7 ; mean $\pm$ SD) and low in the SOL muscle (16.4 ± 1.7) (Fig. 1). The diaphragm was intermediate between the EDL and SOL muscles (34.3 ± 1.6). The concentrations in the masticatory muscles were also intermediate between the EDL and SOL muscles (45.2 ± 4.0 in the digastric, 43.6 ± 3.5 in the temporalis, and 31.1 ± 2.2 in the masseter). The concentration in the lingual muscle (20.2 ± 2.4) was comparable to that in the SOL muscle.

The concentration of S-100a₀ (ng/mg) in the EDL and SOL muscles was 42.6 ± 3.0 and 66.7 ± 3.8 , respectively (Fig. 1). The concentrations in the masticatory muscles and the diaphragm were much lower than in other muscles (17.1 ± 3.6 in the temporalis, 16.4 ± 3.4 in the digastric, 12.4 ± 1.9 in the masseter, and 16.4 ± 1.9 in the diaphragm). The concentration in the lingual muscle (33.2 ± 2.7) was rather similar to that in the EDL muscle.

The concentration of CKM (μ g/mg) in the EDL

and SOL muscles was 172.0 ± 4.1 and 72.7 ± 4.6 , respectively (Fig. 1). The diaphragm was intermediate between these two muscles, but relatively close to the SOL muscle (98.8 ± 3.8). The masticatory muscles (181.3 ± 7.1 in the temporalis, 162.6 ± 7.8 in the digastric, and 147.1 ± 6.7 in the masseter) were comparable to the EDL muscle in the CKM concentration. The concentration in the lingual muscle was lowest (54.3 ± 4.0).

The concentration of CKB (ng/mg) in the EDL and SOL muscles was 209.4 ± 8.7 and 359.5 ± 10.5 , respectively (Fig. 1). The diaphragm was intermediate, but closer to the EDL muscle (255.8 ± 8.3). The digastric muscle was intermediate between the SOL and EDL muscles (282.5 ± 8.7), whereas the jaw-closers were comparable to the EDL muscle (202.5 ± 7.4 in the masseter, and 187.5 ± 8.7 in the temporalis). The concentration in the lingual muscle was extremely high (900.5 ± 13.8).

DISCUSSION

Enolase is a glycolytic enzyme, and has a dimeric structure composed of three kinds of subunit, α , β and γ (5, 24). The $\beta\beta$ -form are present at a high concentration in the skeletal muscles, whereas the $\alpha\beta$ -form is abundant in the heart (15) and the $\alpha\gamma$ - and $\gamma\gamma$ -forms are rich in the nervous tissue (12).

S-100 protein is found in three forms; S-100a₀, S-100a, and S-100b. Each is a dimer with subunit composition of $\alpha\alpha$, $\alpha\beta$, and $\beta\beta$, respectively. S-100a₀ is rich in the heart and striated muscles (13), especially in the slow twitch muscle fibers (6). S-100a and S-100b are found at a high level in the brain (17) and adipose tissues (28).

Creatine kinase in the cytoplasm is a dimeric molecule and has three forms of isozymes (4). CKMM is abundant in skeletal muscles, and CKMB in cardiac muscles (14). CKBB is an acidic protein and is rich in the brain and embryonic skeletal muscles. Soon after birth, the BB-form in skeletal muscles is replaced by the MM-form (3). The fast-twitch muscle in adult develops from the slow-twitch muscle of embryo (2).

The fiber type composition and the speed of contraction of the muscles in the oral region have been studied most often in cats (7, 8, 10, 29). In rats (9, 30, 33), humans (25, 32), guinea pigs (18), and other animals (9, 30), much less information is available. These studies point out that there is marked species difference in the properties of the oral muscles.

The intrinsic tongue muscle of the cat has been reported to be composed almost exclusively of type II fibers, whereas in the extrinsic tongue muscle the percentage of type I and II fibers is 15–19% and 75–81%, respectively (7). The speed of contraction of the intrinsic tongue muscle of the cat is comparable to that of the fast-twitch muscles in the leg (8). In rats, it has been reported that the tongue has a high mitochondrial content and is composed of muscle fibers of relatively small diameter, much smaller than those in the SOL muscle (33). The contraction velocity of the rat lingual muscles has not been measured. The concentrations of β -enolase and CKM in the lingual muscle were not much different from those in the SOL muscle. However, with respect to the other two muscle proteins, these two muscles were definitely different and the concentration of S-100a₀ in the lingual muscle was rather similar to that in the EDL muscle.

In the masticatory muscles of the cat the predominant fibers are type II and their contraction time is very short (superfast) (10, 29). In rats, the masseter and temporalis muscles are composed exclusively of type I fibers, whereas in the digastric muscle some type I fibers appear (9). In terms of the mechanical property, the masseter muscle of rats belongs to the fast muscle (9). The concentrations of four muscle proteins in the masticatory muscles were relatively similar to each other. The concentrations of CKM and CKB were close to those in the EDL muscle. However, the masticatory muscles differed markedly from the EDL muscle in the concentration of S-100a₀ and β -enolase.

The histochemical and contractile properties of the diaphragm of rats have been reported to be intermediate between the EDL and SOL muscles (21). The concentrations of β -enolase, CKM, and CKB were also intermediate between the latter two muscles. However, the concentration of S-100a₀ in the diaphragm was extremely low and comparable to that in the masticatory muscles.

It is concluded that, when assessed from the concentration of the four muscle proteins, 1) the masticatory muscles are relatively similar to each other, 2) the lingual muscle differs widely from the masticatory muscles, and 3) expression of different muscle proteins is controlled differentially in individual muscles and is not necessarily correlated with the conventional classification of fiber types.

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